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Swelling, Mechanical and Antimicrobial Studies of Ag/P(HEMA/IA)/PVP Semi-IPN Hybrid Hydrogels

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A simple and fast approach to the design and production of new hybrid polymeric biomaterials with silver particles is presented in this work. Silver/semi-interpenetrating network hybrid hydrogels (Ag/semi-IPNHHs) were prepared through an optimized solution crosslinking copolymerization of 2-hydroxyethyl methacrylate (HEMA) and itaconic acid (IA), in the presence of PVP, a silver salt and a reducing "green" agent (Ag/P(HEMA/IA)/PVP). PVP was chosen due to its protective, reduction, and nucleation properties in the production of metal particles. The structure of the Ag/semi-IPNHH was characterized by Fourier transform infrared spectroscopy (FTIR). The presence of silver and PVP in the network was confirmed by FTIR spectra. The results obtained by dynamic mechanical analysis (DMA) showed good mechanical properties for all samples. The swelling studies of Ag/P(HEMA/IA)/PVP were conducted in the temperature range of 25–55 °C, in the buffer of pH 7.40. The Ag/semi-IPNHH showed temperature-sensitive swelling properties, with the lower critical solution temperature (LCST) values in the physiologically interesting interval. The antimicrobial activity of the samples was tested using *E. coli*, *S. aureus* and *C. albicans* pathogens. It was concluded that the antimicrobial potential depends on the hydrogel's composition and the type of microbes

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1. Introduction

Recent trends have demonstrated that hydrogels are very promising as "reactors" for *in situ* introduction of metal particles, and this strategy has brought up a new concept in hybrid or composite systems in materials and engineering science. This methodology is noteworthy for the possibility to control the size and morphology of the particles by varying the amount of monomer, crosslinker, and functionality of gel networks. A few studies that deal with metal particles, especially silver, gold, and copper, have exhibited their antimicrobial activity on microorganisms. Silver particles are considered as nontoxic and environmentally friendly antibacterial materials, but due to their poor binding to surfaces, their utility is restricted. Therefore, embedding of polymer-stabilized particles in hydrogel networks is an outstanding approach for biomedical applications [1–4].

Poly(vinyl pyrrolidone) (PVP) is a well-known biologically and environmentally friendly polymer and has been developed for biomedical applications such as artificial organs, wound dressing, artificial skin, and cardiovascular devices [5, 6]. PVP is one of the most widely used polymers in medicine because of its solubility in water and its extremely low cytotoxicity. By introduction of PVP, materials with higher hydrophilicity are obtained, that generally have excellent biocompatibility with living tissues [7]. PVP is also a very good choice for making hydrogels. It is probably the most effective polymer used to increase water uptake ability of 2-hydroxyethyl methacrylate (HEMA) hydrogels [8, 9]]. It has been pointed out that polymers or copolymers containing carboxylic acid groups (such is itaconic acid) are highly desirable in biomaterials, as such groups represent functionality useful for yielding a wide variety of biomedical products. Hydrogels have the available carboxylic acid functional groups, which can be used in any further incorporation of drugs or other bioactive agents [10–12]. The swelling of hydrogels bearing weak acid moieties depends on the acid content in the hydrogel, as well as on several other variables such as the crosslinking density, pH, and ionic strength [13–17].

In this work we report the results obtained for silver/semi-IPN hybrid hydrogels based on 2-hydroxyethyl methacrylate and itaconic acid, with poly(vinyl pyrrolidone) as the interpenetrating polymer designed as a new antimicrobial biomaterial. Ag/P(HEMA/IA)/PVP samples were characterized by their structural characteristics, swelling and mechanical properties as well as by their antimicrobial performances.

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2. Experimental

2.1. Materials

2-Hydroxyethyl methacrylate (HEMA) (Aldrich, Germany), freshly distilled, itaconic acid (IA) (Fluka, Germany), poly(vinyl pyrrolidone) (PVP) (average MW of 360000, Aldrich), the silver nitrate salt (Fluka), and ascorbic acid (Aldrich) were the reactants used in this study. Ethylene glycol dimethacrylate (EGDMA) (Aldrich), as crosslinking agent, potassium persulfate (KPS) (Fluka), as initiator, and N, N, N′, N′-tetramethylethylene diamine (TEMED) (Aldrich), as activator, were used in all polymerizations performed in a mixture of water/ethanol solution. Potassium hydrogen phosphates (KH_2PO_4 and K_2HPO_4) (La Chema, Czech Republic) were used for buffer preparations. Demineralized water was used for all copolymerizations and the preparation of the buffer solution.

2.2. Preparation of Ag/semi-IPNHHs

The Ag/P(HEMA/IA)/PVP samples were prepared by free radical polymerization/crosslinking and then reduction in water/ethanol solution. Before polymerization, the components: HEMA, IA, PVP and the crosslinker were sequentially added into the water/ethanol mixture, under stirring. After that, solution of $AgNO_3$ and ascorbic acid was added, and the reaction mixture was purged with nitrogen in order to remove the dissolved oxygen from the reaction solution. To initiate the polymerization, KPS and TEMED were added into the reactive system. Then the reactive solution was quickly poured into a glass mould. The polymerization was carried out at 50 ℃ for 24 h. After the reaction, the hydrogel was cut into discs (10 mm in diameter) and immersed in water, which was repeatedly changed for 72 h to remove any residual reactants. Mole fractions (%) of P(HEMA/IA)/PVP samples are following: 93/5.0/2.0 (P(HEMA/IA)/PVP-2), 90/5.0/5.0 (P(HEMA/IA)/PVP-5), 85/5.0/10.0 (P(HEMA/IA)/PVP-10). The initiator, activator and crosslinker are added to a monomer feed mixture in the amount of 0.25, 0.25 and 0.5 mol%, respectively, with respect to the total moles of monomers. Concentration of $AgNO_3$ solution is 10^{-3} M.

2.3. FTIR spectroscopy

Dry gels were crushed into powder and mixed with potassium bromide (Merck, Germany, IR spectroscopy grade) in the proportion 1:100, and dried at 40 ℃. The mixture was compressed to a 12 mm semi-transparent disk by applying a pressure of 65 kN (Pressure gauge, Shimadzu) for 2 min. FTIR spectra over the wavelength range 4000-700 cm^{-1}, with a resolution of 4 cm^{-1}, were recorded using a FTIR spectrometer (BOMEM Michelfan MB-102 FTIR).

2.4. Dynamic-mechanical analysis (DMA)

Strain-frequency sweeps were performed on hydrogel discs using a Rheometrics 605 mechanical spectrometer, with parallel plates geometry (25 mm in diameter). The shear modulus was measured as a function of frequency (ω), from 0.1 to 100 rad/s, at 37 ℃.

2.5. Swelling study

Dynamic swelling measurements were performed in a pH 7.40 buffer solution (simulated physiological fluid) in the temperature range of 25-55 ℃. Swollen gels were removed from the swelling medium at regular intervals, dried superficially with filter paper, weighed and placed in the same bath. The measurements were continued until the equilibrium was reached. The amount of solution absorbed was monitored gravimetrically. The equilibrium degree of swelling (q_e) was calculated as follows:

$$q_e = (M_e - M_0)/M_0 \quad (1)$$

where M_e is the weight of the swollen hydrogel at equilibrium and M_0 is the weight of the dry gel [18, 19]. All the swelling experiments were performed in triplicate.

2.6. Antimicrobial activity assay

The antimicrobial agent loaded hydrogel samples were inoculated in tubes with saline solution (9 ml). The selected indicator microorganisms were *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), and *Candida albicans* (ATCC 24433). The number of bacterial cells was ca 10^4 ml^{-1} for all strains. The tests were performed in three replicas and the results calculated as a mean value. The tubes were placed into a shaker (120 rpm) and thermostated in the water bath at 37 ℃, to assure better contact of tested hydrogels and bacterial cells. For this purpose, the sterile Petri plates were inoculated with an aliquot (100 μl) of saline solution with the tested hydrogels and bacterial cultures and overlaid with melted TSA. After solidification of agar and the incubation at 37 ℃ for 24 h, counting of the visible colony was performed.

3. Results and discussion

The process of formation of silver particles in the semi-IPNs is presented in Fig. 1.

3.1. FTIR spectra of Ag/P(HEMA/IA)PVP

FTIR spectroscopy was used to characterize the structure of silver/semi-IPN hybrid hydrogels. The spectral characteristics of Ag/P(HEMA/IA)/PVP samples are presented in Fig. 2. The peaks at 1710 and 1677 cm^{-1} are due to the different content and different microenvironment of PVP. The absorption bands at 1595, 1465 and 1414 cm^{-1} were assigned to the vibration of the pyridine ring, and the band at 2900 cm^{-1} to the stretching vibration of the aliphatic CH_2. The bands characteristic of HEMA and IA components are mainly the C=O group at 1730 cm^{-1} and O–H stretching at 3500 cm^{-1}.

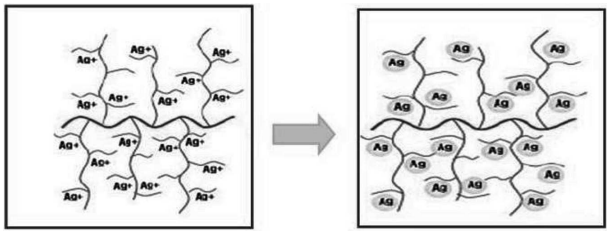

Fig. 1. The scheme presenting formation of silver particles in semi-IPNs.

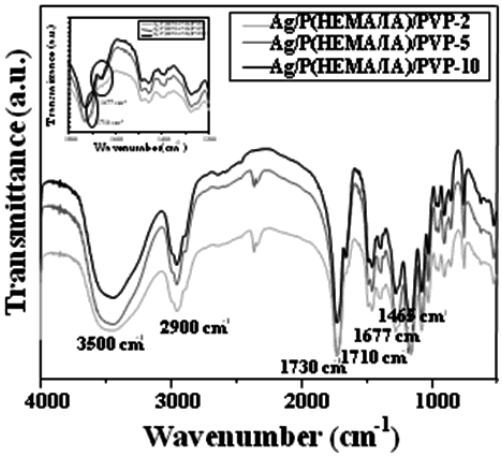

Fig. 2. FTIR spectra of Ag/P(HEMA/IA)/PVP.

3.2. Mechanical properties of Ag/P(HEMA/IA)PVP

The suitability of a hydrogel for biomedical applications is governed to a large extent by its network structure, which is defined during syntheses by the monomer and crosslinking agent concentration, monomer ratio (in copolymers), solvent used, temperature and gel preparation technique. For non-biodegradable applications, it is essential that the carrier gel matrix maintains its physical integrity and mechanical strength. The mechanical properties of the hydrogel are, therefore, an important issue when designing a therapeutic system for medical and pharmaceutical purposes in drug delivery systems. The strength of the matrix can be increased by incorporating adequate comonomers or increasing the degree of crosslinking [20]. However, for each system there is an optimal degree of crosslinking, as the transport properties of drugs in each type of gel vary according to the structure and morphology of the network. Elasticity of the gel should be pronounced to give flexibility to the polymer chains, which facilitates movement of incorporated bioactive agent. Thus, a compromise between the mechanical strength and flexibility is necessary for appropriate use of these materials.

We measured the shear moduli of silver/semi-IPN hybrid hydrogel samples in order to evaluate their mechanical properties. Figure 3 shows the variation of shear storage modulus with frequency for the Ag/P(HEMA/IA)/PVP hydrogels in the relaxed state (G'_{relax}). The values of the modulus were in the range of 2.56-4.43 kPa. It is evident that G'_{relax} depends on the PVP content in silver/semi-IPNHHs. A small amount of PVP incorporated in silver/semi-IPNHHs improved their mechanical performances. The value of G'_{relax} increases as the PVP content increases and the highest value was found for the sample with 10 mol% of PVP. The Ag/P(HEMA/IA)/PVP-2 hydrogel showed the lowest G'_{relax} value. This behaviour is probably due to the ability of PVP, as interpenetrant, to improve the mechanical strength of polymer networks due to the formation of H-bonds, as additional physical crosslinks [21].

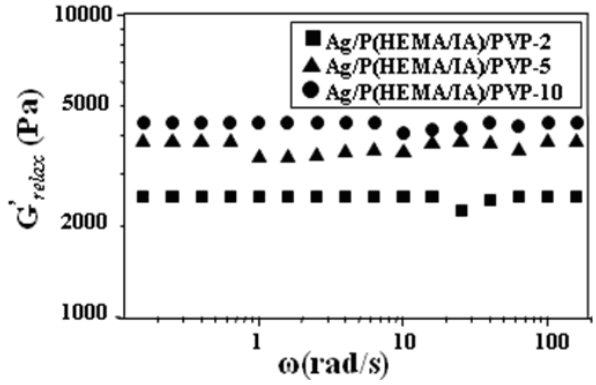

Fig. 3. Shear storage modulus (G'_{relax}) for the Ag/P(HEMA/IA)/PVP in relaxation state, at 37 ℃.

3.3. Temperature sensitivity of Ag/P(HEMA/IA)/PVP

The equilibrium degrees of swelling (q_e) vs. temperature of Ag/semi-IPN hybrid hydrogels at pH 7.40 are shown in Fig. 4. It can be seen that all samples show the temperature-sensitive swelling behaviour. The q_e values drastically decrease above around 41 ℃, which is interpreted as the lower critical solution temperature (LCST) of the Ag/P(HEMA/IA)/PVP hybrid hydrogels. Ag/P(HEMA/IA)/PVP hybrid hydrogels have the LCST at physiologically important temperatures around 41 ℃. LCST values are 42.4, 41.0, and 40.5 ℃ for samples with 2, 5, and 10 mol% of PVP, respectively. When the temperature is below the LCST, the polymer exists in a swollen, hydrophilic state. However, as the temperature is raised above the LCST, the polymer goes through an abrupt conformational rearrangement, resulting in a collapsed, hydrophobic state. The conformational change is a result of the break-up of the network of hydrogen-bonded water surrounding the hydrophobic segments of the polymer chain. This phase transition is more pronounced for the samples with higher PVP content (Table 1). With the change in the PVP content in semi-IPN hybrid hydrogels, the LCST temperature can be varied and adjusted to the value required for the biomedical application in case.

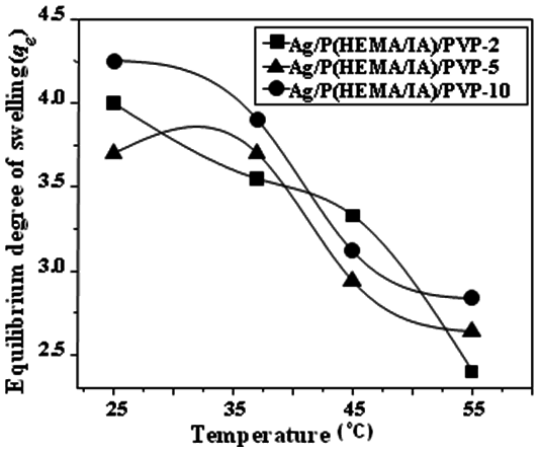

Fig. 4. Temperature-sensitive swelling of the Ag/P(HEMA/IA)/PVP.

3.4. Antimicrobial activity of Ag/P(HEMA/IA)PVP

Antimicrobial activity is a very valuable property for biomedical applications [22–25]. Introduction of antimicrobial agents in hydrogels is important to prevent infection and to protect the hydrogel implants and devices from infection during their use. A study of antimicrobial activity of silver/semi-IPN hybrid hydrogels (Ag/P(HEMA/IA)/PVP) was made to examine the efficacy of newly designed hybrid polymeric biomaterials towards *S. aureus*, *C. albicans*, and *E. coli* pathogens. According to the obtained results presented in Fig. 5, it is evident that the antimicrobial activity depends on the PVP content. The best sensitivity was obtained for the samples tested for antimicrobial activity against the yeast *C. albicans*, one of the most commonly encountered human pathogens, causing a wide variety of infections, ranging from mucosal infections in generally healthy persons to life-threatening systemic infections in individuals with impaired immunity. The reduction in the number of cells for the yeast was in the range of 85-95 % (Fig. 5). A slightly lesser antimicrobial efficacy of hydrogels was obtained for the Gram-positive bacteria *S. aureus*, where a reduction in cell number was about 70 % after two hours of exposure, for the sample with the highest PVP content (Fig. 5). The samples examined had the least antimicrobial activity against the Gram-negative bacteria *E. coli*, where a reduction in cell number was below 16 % (Fig. 5). Additionally, there is an influence of the time of exposure on the potency of antimicrobial activity of Ag/P(HEMA/IA)/PVP. It was observed that the reduction in the number of cells varies with time for all samples and all microbial cultures. Silver affinity (binding) for carbonyl groups of PVP is high. Therefore, the silver concentration is proportional to the PVP content in hydrogels. Generally, it could be said that hybrid hydrogels containing higher content of PVP showed better antimicrobial activity.

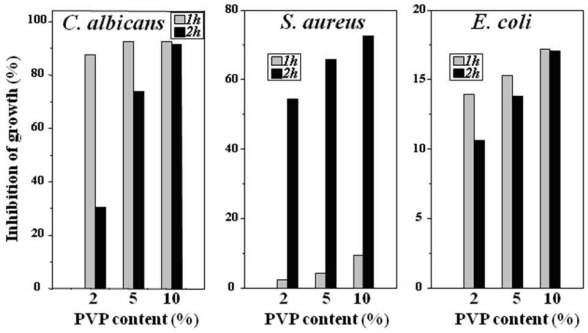

Fig. 5. Antimicrobial activity of Ag/P(HEMA/IA)/PVP for different PVP content.

4. Conclusion

In this work, new silver/semi-IPN hybrid hydrogels were prepared by radical copolymerization/crosslinking of 2-hydroxyethyl methacrylate, itaconic acid, poly(vinyl pyrrolidone), and silver salt, followed by a reduction of the silver ion. FTIR spectroscopy measurements confirmed the incorporation of PVP and Ag in the semi-IPN hybrid hydrogels. Swelling studies revealed the temperature sensitivity of all samples. LCST are in the range of physiologically interesting temperatures. It is perceived that the antimicrobial activity of the Ag/P(HEMA/IA)/PVP depends on the PVP moiety, the type of microbes and the exposure time. The best efficacy was obtained for Ag/P(HEMA/IA)/PVP tested for antimicrobial activity against the yeast *C. albicans*. A slightly lesser antimicrobial efficacy of hydrogels was obtained for the Gram-positive bacteria *S. aureus*. Due to their swelling and antimicrobial properties, smart silver/poly(2-hydroxyethyl methacrylate/itaconic acid)/poly(vinylpyrrolidone) hybrid hydrogels show the potential to be used in the field of biomedicine. A combination of the temperature sensitivity of the obtained hybrid hydrogels (i.e. identified LCST values in the physiologically important range of temperatures) and good antimicrobial properties leads to hybrid systems with advanced performances in biomedical applications. From our results, it turns out that Ag/P(HEMA/IA)/PVP hybrid hydrogels can be used for regeneration of injured tissues.

Acknowledgments

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